Spatial Phenotypic Signatures: A Novel Biomarker Class

Spatial Phenotypic Signatures measure the interactions and cell densities of tumor and immune cells in the tumor microenvironment (TME). The resulting cell-by-cell maps of the TME provide deeper insights into the tumor-immune biology, that could inform treatment response.

A recent meta-analysis of more than 8000 samples, published in JAMA Oncology1, showed that spatial phenotyping, enabled by multiplex immunofluorescence (mIF), more accurately predicts patient response to anti-PD-1/PD-L1 therapy than other biomarker assays including PD-L1 IHC, tumor mutational burden (TMB), and gene expression profiling (GEP).


THE PHENOIMAGER SOLUTION

Measure Spatial Phenotypic Signatures, at Scale

FAST
Rapid whole-slide scans in less than 20 minutes

ACCURATE
Proprietary Multispectral Imaging (MSI) technology enables quantitative and accurate phenotyping

INTEGRATED
Comprehensive multiplex imaging solution optimized from sample to result

EFFICIENT
Save precious samples by detecting multiple biomarkers in a single section

Proven technology with over 400 peer-reviewed publications

*Patent pending
**AN INTEGRATED END-TO-END SOLUTION**

PhenoImager Workflow

The discovery and validation of Spatial Phenotypic Signatures requires a solution that easily integrates staining, imaging, and analysis using existing workflows while providing accuracy and reproducibility.

**SHIFTING THE PARADIGM: VISUAL IHC TO QUANTITATIVE SPATIAL PHENOTYPING**

Akoya’s Opal™ reagents are optimized for reliable Multispectral Imaging (MSI) making multiplex results accessible to anyone who works with standard IHC in formalin-fixed, paraffin-embedded (FFPE) tissue.

**Opal’s Tyramide Signal Amplification (TSA)-based detection provides:**

- Up to 8 markers on a single tissue – manual or automation
- Improved sensitivity (10 to 100-fold)*
- Higher dynamic range up to 4 logs*
- Reduced reagent consumption

*Compared to chromogenic IHC

**STAIN**
Whole-slide staining of tissues using pre-designed or custom antibody panels.

**IMAGE**
Rapid whole-slide image acquisition with touchless walkaway automation.

**ANALYZE**
Visualize and interpret using Akoya’s software suite or open-source solution.

**DOWNLOAD TECH NOTE AT:** akoyabio.com/opal-panels
Conventional imaging systems utilize narrow bandpass filters that capture only a snapshot (typically the peak) of each fluorophore’s emission spectrum. Accurate analysis is often complicated with issues such as tissue autofluorescence and spectral overlap.

Figure on the right shows fluorophore emission spectra with representative examples of bandpass filters (colored boxes).

WHOLE-SLIDE SCANNING REVEALS COMPLEX BIOLOGY IN MINUTES

The PhenoImager Fusion and HT instruments are the fastest quantitative imaging platforms enabling 6-plex whole-slide scans in less than 20 minutes. Powered by proprietary MSI technology, the PhenoImager instruments provide rapid and accurate spatial phenotyping, at scale.

**PhenoImager™ Fusion**
- **Spatial Signatures within Reach**
- STANDARD THROUGHPUT: 4 slides per run, 900+ slides per week
- FLEXIBLE: Integrates with PhenoCyliner™ for ultrahigh-plex imaging (100+ biomarkers)

**PhenoImager™ HT**
- **Spatial Signatures at Scale**
- ULTRA HIGH THROUGHPUT: 80+ slides per run, 300+ slides per week with continuous loading
- AUTOMATED: Touchless automation technology

DATA ACCURACY WITH HIGHER PLEXING
Akoya’s Proprietary Multispectral Imaging (MSI) Technology

Conventional imaging systems utilize narrow bandpass filters that capture only a snapshot (typically the peak) of each fluorophore’s emission spectrum. Accurate analysis is often complicated with issues such as tissue autofluorescence and spectral overlap.

Rather than using only a small portion of each fluorophore’s emission spectrum, Akoya’s MSI Technology allows users to gather the entire emission signature to achieve robust spectral unmixing. This makes each fluorophore’s signal truly distinct from those of other fluorophores and enables the clear separation of signals, as well as the removal of autofluorescence.

Akoya’s MSI technology applied to stained fluorescent images can isolate autofluorescence, increasing accuracy of phenotyping up to 50%.

*Patent pending*
FAST, ACCURATE DATA GENERATION

From Images to Phenotypes to Signatures

Akoya’s biomarker discovery tools combine the latest technologies with algorithms and intuitive, easy-to-use interfaces, giving you the power to make new, exciting discoveries from your data.

The PhenolImager Advanced Tissue Image Analysis Software Suite

Phenochart™
Whole-slide contextual viewer enabling viewing and annotation

inForm™
Patented automated tissue analysis software for spectral unmixing, segmentation, and phenotyping

phenoptrReports
Powerful analytical tool to analyze spatial relationships

SPATIAL SIGNATURE STANDARDIZATION

CASE STUDY

The First Multi-Institutional Analytical Demonstration of a Spatial Biology Workflow

The MITRE Study established the high reproducibility and robustness of Akoya Biosciences’ PhenoImager platform for spatial phenotyping in clinical and translational research.

CASE STUDY

Astronomy Meets Pathology for Immunotherapy Research

Investigators at Johns Hopkins University (JHU) took a novel approach to developing spatial phenotypic signatures for accurate prediction of immunotherapy response, combining sky mapping algorithms with Akoya’s cutting-edge PhenolImager platform.

LEARN ABOUT WORKFLOW VALIDATION: akoyabio.com/mitre-validation

LEARN HOW TO DEVELOP A SPATIAL SIGNATURE: akoyabio.com/astropath-signature

DOWNLOAD TECH NOTE AT: akoyabio.com/advanced-analysis

Correlation across sites

60% 80% 100% 120%

0% 20% 40% 60% 80% 100% 120%
Translate your discoveries into actionable spatial phenotypic signatures with our connected ecosystem of solutions.

CASE STUDY
Spatial Analysis of Cellular Neighborhoods

In a pioneering study utilizing the PhenoCycler system, Dr. Garry Nolan's lab at Stanford University developed a novel analysis framework to study tissue biology at two levels—the distinct regions of the tissue and the cell types present in these regions.

SEE THE FULL STUDY: akoyabio.com/cellular-neighborhoods

The PhenoCycler™-Fusion Solution
Spatial Discovery at Scale
Rapid whole-slide imaging of 100+ biomarkers

The PhenolImager Solution
Spatial Signatures at Scale
Rapid whole-slide imaging of 6 biomarkers, 300+ slides per week
## MULTISPECTRAL IMAGING FOR EVERY LAB

<table>
<thead>
<tr>
<th></th>
<th>PhenolImager™ Fusion</th>
<th>PhenolImager™ HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dimensions (W × D × H in)</strong></td>
<td>25&quot; × 20&quot; × 26&quot;</td>
<td>30&quot; × 28&quot; × 28&quot;</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>~120 lbs./54.4 kg</td>
<td>~187 lbs./85 kg</td>
</tr>
<tr>
<td><strong>Tissue Format</strong></td>
<td>Whole-slide, Tissue Microarray and Tissue Sections</td>
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</tr>
<tr>
<td><strong>Detection Method</strong></td>
<td>Fluorescence, Brightfield</td>
<td>Fluorescence, Brightfield</td>
</tr>
<tr>
<td><strong>Light Source</strong></td>
<td>LED</td>
<td>LED</td>
</tr>
<tr>
<td><strong>Multispectral Range</strong></td>
<td>440 nm – 780 nm</td>
<td>440 nm – 780 nm</td>
</tr>
<tr>
<td><strong>Speed (1.5cm X 1.5cm)</strong></td>
<td>Fluorescence: 18 min; Brightfield: 9 min</td>
<td>Fluorescence: 12 min; Brightfield: 6 min</td>
</tr>
<tr>
<td><strong>Throughput</strong></td>
<td>4 slide</td>
<td>80 slide (with continuous loading technology)</td>
</tr>
<tr>
<td><strong>Multiplexing Capability</strong></td>
<td>Separates up to 7 colors</td>
<td>Separates up to 9 colors, even if overlapping</td>
</tr>
<tr>
<td><strong>Resolution</strong></td>
<td>10X (1.0 μm/pixel), 20X (0.5 μm/pixel) or 40X (0.25 μm/pixel)</td>
<td>10X (1.0 μm/pixel), 20X (0.5 μm/pixel) or 40X (0.25 μm/pixel)</td>
</tr>
<tr>
<td><strong>Image Analysis Software</strong></td>
<td>inForm® and phenoptrReports</td>
<td>inForm® and phenoptrReports</td>
</tr>
<tr>
<td><strong>File Format</strong></td>
<td>Akoya Biosciences' whole-slide scan image (.QPTIFF)</td>
<td>Akoya Biosciences' whole-slide scan image (.QPTIFF), LCTF enabled; Multispectral images (.im3), color images (.JPEG, .BMP, .PNG)</td>
</tr>
<tr>
<td><strong>Automation</strong></td>
<td>Touchless, with walk-away image acquisition</td>
<td>Touchless, with walk-away image acquisition</td>
</tr>
<tr>
<td><strong>Power Requirements</strong></td>
<td>100-240VAC, 50/60 Hz</td>
<td>100-240VAC, 50/60 Hz</td>
</tr>
</tbody>
</table>

*Measurements are approximations and could vary slightly for the final shipments*